THE AGGLUTINATION OF STARFISH SPERM BY FERTILIZIN[1]

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Agglutination of starfish sperm by specific egg water (supernatant sea water from egg suspensions) has never been clearly demonstrated. Glaser (1914) and Woodward (1918) reported a strong agglutination of *Asterias forbesii* sperm by homologous egg water, but Just (1930) was unable to confirm this work. Attempts to demonstrate agglutination of sperm by egg water in other species of starfish have failed. Thus Loeb (1914) observed no reaction in *Asterias* (probably *Pisaster*) *ochraceus*, and Tyler (1941) had a similar result with *Patiria miniata*. From this it might appear that fertilizin is not present in starfish egg water. However, Tyler found that treatment of *Patiria* sperm with egg water lowered the fertilizing power of the sperm. Tyler (1941, 1942) interpreted this as support for his view that fertilizin may exist naturally in a non-agglutinating "univalent" form. An individual molecule of such univalent fertilizin should have but one combining group capable of reacting with groups (antifertilizin) on the sperm surface. On the basis of the Marrack-Heidelberger (1938) lattice theory, univalent fertilizin should therefore combine with but not agglutinate these cells. Tyler suggests that such univalent fertilizin may be present quite generally in forms showing no agglutination of sperm by egg water. He therefore supports the belief held by Lillie (1919) and Just (1930) that fertilizin occurs universally.

In view of the concept of univalent fertilizin and the provisional status of the starfish with respect to sperm agglutination by egg water, it is of some interest that sperm of certain starfish agglutinate when mixed with homologous egg water and an "adjuvant." The first adjuvant found was lobster (*Panulirus*) serum. The agglutination reaction was discovered accidentally in the course of studies on the natural agglutinins in lobster serum (Tyler and Metz, 1944). In an attempt to separate natural agglutinins for *Patiria* eggs and sperm, the serum was treated with eggs and then titrated for sperm agglutinins. The treatment with eggs increased the sperm agglutinin titer several fold. Investigation of this unexpected result showed that sperm absorbed lobster serum (freed of natural sperm agglutinins), when mixed with *Patiria* egg water, agglutinated *Patiria* sperm. Tests on other material showed the presence of adjuvant in hen's egg white. A preliminary report (Metz, 1944) on this work has already appeared. The studies confirm Tyler's view that fertilizin is present in *Patiria* egg water. However, the experiments indicate that this fertilizin is multivalent. Data are given which suggest that normal *Patiria* sperm is "univalent" with respect to exposed antifertilizin groups, but that more of these groups are "exposed" by the adjuvant.

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Material and Methods

The Pacific webbed star, *Patiria miniata*, was used as standard material. The Pacific star *Pisaster ochraceus*, the Pacific sand star *Astropecten* sp. and the Atlantic *Asterias forbesii* were used in confirmatory and specificity tests.

Egg and sperm suspensions were prepared from ripe extirpated gonads. These organs were minced in a measured volume of sea water and then filtered through bolting cloth to remove the gonadal tissue. The difference in volume of the filtrate and the sea water initially added gives the volume of "dry" (undiluted) material. Egg and sperm dilutions were reckoned from this "dry" volume. Egg water solutions were obtained by drawing off the supernatant from standing egg suspensions (25–50 per cent of dry eggs in sea water), or by heating such suspensions and filtering or centrifuging off the eggs.

Lobster (*Panulirus interruptus*) serum was obtained by drawing blood from the heart and allowing it to clot. After syneresis the serum was drawn off. Since *Panulirus* serum contains natural heteroagglutinins for sperm of various organisms (Tyler and Metz, 1944) including *Patiria, Pisaster* and *Astropecten*, it is impractical to use the untreated serum. By absorption with *Patiria* sperm the natural agglutinins for *Pisaster* and *Astropecten* as well as *Patiria* sperm can be removed. Such absorbed serum was used as the adjuvant for sperm of all three species. For reasons of economy both in material and time, hen's egg white was used as the adjuvant in the later experiments. This material was made isotonic by adding one volume of concentrated (1.73 ×) sea water. It was then diluted to 20 per cent with normal sea water and filtered to remove the mucin, chalazae and other insoluble material. This diluted egg white was usually heated to 100° C. and filtered or centrifuged since this procedure increased its activity several fold. Hen's egg white does not contain natural agglutinins for *Patiria, Pisaster* or *Astropecten* sperm. Thus, initial absorption with starfish sperm was not necessary.

Assays of unknown egg water were made by diluting the unknown solution in twofold steps with sea water and then adding constant amounts of adjuvant-treated sperm to each dilution of unknown egg water. Adjuvant was titrated in a similar manner. However, when titrating adjuvant, constant amounts of sperm suspension were added to the dilutions of unknown adjuvant. Subsequently, constant amounts of egg water were added to each adjuvant dilution. Less satisfactory results are obtained if any other order of mixing is employed in this test. In all cases the presence or absence of agglutination was determined by microscopical examination one to several minutes after mixing. Titers were recorded as the highest dilution of unknown showing agglutination of the test sperm.

The apparatus and methods used in ultraviolet irradiation have been described in a previous article (Metz, 1942).

Activation and Agglutination of Starfish Sperm

In these studies no definite agglutination of *Patiria, Pisaster, Astropecten* or *Asterias* sperm was observed following the addition of homologous egg water. However, *Patiria* sperm suspensions frequently appeared "granular" after this treatment. These microscopic "granules" consisted of two or three sperm fixed together and represent a plus-minus agglutination reaction. Various devices such

as centrifugation were employed in an attempt to bring this reaction to a distinct agglutination, but all of them failed.

The starfish sperm in dilute (0.5 to 1.0 per cent) sea water suspension were virtually immobile. The cells did not respond to treatment with fresh sea water (lowering CO_2 tension) or with homologous egg water. Starfish sperm thus differ from *Arbacia* and *Nereis* sperm which become more active when diluted with sea water (Lillie, 1913; Just, 1930), and from *Arbacia* (Lillie, 1913), *Strongylocentrotus* (Tyler, 1939) and *Megathura* sperm (Tyler, 1940) which become intensely motile when mixed with homologous egg water.

Patiria, Pisaster and *Astropecten* sperm, although refractory to treatment with sea water and egg water, nevertheless became intensely active when treated with isotonic hen's egg white or the serum of the lobster (*Panulirus*), fish (*Crassius*), hen, or rabbit. Furthermore, adjuvant-treated sperm of these starfish agglutinated strongly on addition of homologous egg water. *Asterias* was tested on three successive seasons. The sperm became intensely active when treated with isotonic hen's egg white. Weak agglutination sometimes occurred after addition of homologous egg water to the sperm egg white suspension. Unfortunately, the agglutination was so weak and occurred so irregularly that quantitative studies could not be made.

In *Patiria* the agglutination resulting from treatment of sperm with adjuvant and egg water was exclusively head to head. Each clump consisted of a central mass of sperm heads tightly bound together, and a peripheral region of free tails which projected out radially from the central mass of heads. *Patiria* thus differs from *Megathura,* since sperm of the latter agglutinate tail to tail as well as head to head (Tyler, 1940). The clumped *Patiria* sperm soon became immobile even though the free sperm remained active for an hour or more. The spontaneous reversal of agglutination so characteristic of the sea urchin occurred to a limited extent only after the free sperm had become inactive.

Properties of Patiria Fertilizin

Fertilizin may be defined by the following properties: (1) it combines with (but does not necessarily agglutinate) sperm, (2) it is highly specific in this reaction, and (3) it is obtained primarily from eggs. Studies on the role of egg water in the agglutination of treated sperm show that *Patiria* egg water has these properties.

Absorption of Patiria egg water by sperm. A direct combination between sea urchin fertilizin and sperm may be demonstrated by absorption of egg water with sperm, or by neutralization of egg water with appropriate sperm extract (Lillie, 1913; Frank, 1939). Similarly, it may be shown that sperm-absorbed *Patiria* egg water will no longer agglutinate treated sperm. Indeed, complete exhaustion of the egg water may be attained even in the absence of adjuvant. In a typical experiment 20 drops of *Patiria* egg water were mixed with 22 drops of concentrated (25–50 per cent) *Patiria* sperm. The mixture was set aside to allow for reaction. Twenty drops of the same egg water were mixed with 22 drops of sea water to serve as a control. After centrifugation the fluid of both tubes was titrated with adjuvant-treated (0.5–1 per cent) sperm. The undiluted absorbed egg water did not agglutinate the sperm, whereas the control unabsorbed egg water clumped the sperm

even at a dilution of $\frac{1}{256}$ of full strength. Other controls showed that sperm without adjuvant were not agglutinated by control or absorbed egg water, or by the adjuvant (*Patiria* sperm-absorbed *Panulirus* serum). Thus a substance (fertilizin) is present in *Patiria* egg water which will combine with specific sperm independently of the adjuvant. The adjuvant is required only for agglutination.

Specificity of starfish fertilizin. The reaction between sperm and fertilizin is characterized by a high order of specificity (Tyler, 1940). Cross tests between *Patiria, Pisaster* and *Astropecten* sperm and fertilizin show that these starfish are not exceptional in this respect. Sperm suspensions of the three species were treated with *Patiria* sperm-absorbed *Panulirus* serum, and then cross tested with the egg waters of the three species. The data are given in Table I.

TABLE I

Specificity of Patiria, Pisaster *and* Astropecten *egg waters*

		Patiria sperm	*Pisaster* sperm	*Astropecten* sperm
Patiria egg + water	adjuvant	+++	±	−
	sea water	±	−	−
Pisaster egg + water	adjuvant	−	+++	−
	sea water	−	++	−
Astropecten egg + water	adjuvant	−	−	±
	sea water	−	−	−
	adjuvant + sea water	−	−	−
	Patiria sperm supernatant	−	−	−

It will be seen that *Patiria* and *Pisaster* egg waters agglutinated only homologous sperm. Thus the species specificity rule holds for these two forms. In this experiment *Pisaster* egg water clumped homologous untreated sperm. This reaction did not occur with predictable regularity. The reaction between *Astropecten* egg water and homologous sperm was doubtful. This may be ascribed to neutralization of the *Astropecten* egg water by the *Patiria* sperm supernatant present in the adjuvant solution. The relationship here is somewhat involved. With the exception of the reaction between *Patiria* sperm supernatant and *Astropecten* egg water, the reactions were species specific.

The source of Patiria fertilizin. Only egg water prepared from suspensions of *Patiria* eggs possessing their normal gelatinous coats agglutinated species sperm in the presence of the adjuvant. Blood from female animals did not have this effect. Thus it may be concluded that a specific substance is obtained from starfish eggs which will react with and under certain conditions agglutinate species

sperm. This then gives clear and direct support to Tyler's (1941) view that fertilizin exists in the *Patiria* egg water.

The Nature of Patiria Fertilizin

Tyler (1941) concluded that *Patiria* fertilizin was univalent for combining groups complementary to sperm. It follows from the lattice theory that such univalent fertilizin must become multivalent to agglutinate the sperm. The adjuvant should then convert the natural univalent *Patiria* fertilizin to a multivalent, agglutinating form. However, another possibility in accord with the lattice theory is that the fertilizin is multivalent but the sperm is normally univalent. The results of the following experiments favor this latter view.

Effect of ultraviolet light on Patiria fertilizin. Sea urchin fertilizin can be converted to the univalent form by proper exposure to heat, enzymes, x-radiation and ultraviolet light (Tyler, 1941; Metz, 1942). Such treated fertilizin will not agglutinate sperm but it will combine with sperm rendering the sperm unagglutinable by untreated fertilizin. To test for the possibility of a similar action, *Patiria* fertilizin was exposed to ultraviolet irradiation. It was found that irradiated *Patiria* fertilizin will not agglutinate adjuvant-treated homologous sperm, and normal fertilizin will not subsequently agglutinate the sperm that has been treated with irradiated fertilizin. Thus it is possible that the natural fertilizin is multivalent and the irradiated material is true univalent fertilizin. The data from a typical experiment are given in Table II.

Table II

Destruction of agglutinating power of Patiria *fertilizin by ultraviolet light and agglutination inhibiting properties of this fertilizin*

Solution	Irradiated fertilizin	Control fertilizin	Irradiated fertilizin + control fertilizin	Irradiated sea water + control fertilizin
Reaction of adjuvant-treated sperm	−	++++	−	++++

Two stender dishes each containing 5 cc. of a *Patiria* fertilizin solution and one dish containing 5 cc. of sea water were irradiated for 220 minutes. The control fertilizin sample was screened from the ultraviolet light by a "noviol C" filter. After the irradiation the control and irradiated fertilizin samples were tested for agglutinin activity by mixing 2 drops of hen's egg white treated sperm (1%) with 2 drops of each fertilizin solution. At the same time 2 drops each of the sperm and irradiated sea water were mixed. It will be seen that the irradiated fertilizin was inactive whereas the control strongly agglutinated the sperm. After this examination one drop of unirradiated test fertilizin was added to the irradiated fertilizin-adjuvant-sperm mixture and one drop to the irradiated sea water-adjuvant sperm. In this test for inhibition of agglutination it will be seen that sperm treated

with irradiated fertilizin did not agglutinate upon subsequent addition of normal fertilizin, whereas the sperm treated with irradiated sea water reacted strongly.

Agglutination of adjuvant-free Patiria sperm by fertilizin. More definite evidence for the multivalent nature of starfish fertilizin was obtained from a study of the effect of adjuvant on sperm. Adjuvant was added to *Patiria* sperm and then removed. Such adjuvant free sperm agglutinated on addition of natural fertilizin. Twenty drops of 0.5 per cent sperm were mixed with 10 drops of isotonic hen's egg white. A control sample consisted of 20 drops of 0.5 per cent sperm plus 10 drops of sea water. Both samples were centrifuged and the packed sperm was resuspended in 20 drops of sea water. Two drops of sperm from each were tested with *Patiria* fertilizin. The control suspension did not react whereas the sperm centrifuged from the egg white agglutinated moderately. The suspensions were recentrifuged and the supernatants tested and found free of adjuvant. The sperm masses were resuspended in 16 drops of sea water after the second centrifugation and tested. The control sperm did not react to fertilizin whereas the sperm previously treated with adjuvant agglutinated weakly.

This experiment was not confirmed with *Astropecten*. *Astropecten* sperm after centrifugation from hen's egg white solution were not agglutinated by homologous fertilizin alone, although this sperm reacted strongly when both egg white and fertilizin were added.

It seems clear then that *Patiria* fertilizin will agglutinate sperm after the adjuvant has been removed from the sperm. It may therefore be concluded that the natural *Patiria* fertilizin is multivalent.

Univalent Sperm

Evidence has just been presented to show that natural *Patiria* fertilizin is multivalent and capable of agglutinating sperm. It follows that the normal sperm is incapable of agglutination. The adjuvant must then convert the sperm to an agglutinating condition.

It seems unlikely that stimulation of the normally immobile sperm to intense activity is of any considerable importance in this adjuvant-fertilizin agglutination of *Patiria* sperm since immunological doctrine does not require motility of cells for agglutination. Thus non-motile bacteria and erythrocytes agglutinate strongly when mixed with specific antibody. Furthermore, heat killed sea urchin sperm agglutinate strongly on addition of fertilizin. However, heat killed *Patiria* sperm did not react when mixed with fertilizin and adjuvant. The deficiency of the normal sperm must then involve the antigenic structure of the cell surface. For agglutination to occur the area of the sperm surface containing groupings complementary to fertilizin must be rather extensive. If this region of the sperm surface were limited in extent and contained but a few or even a single antifertilizin group, the sperm could be considered "univalent" for this particular antigen. Such sperm should not agglutinate when mixed with complementary agglutinin (fertilizin). At best only two or three sperm could clump together. This condition is occasionally observed when untreated *Patiria* sperm and fertilizin are mixed. It has been described as the "granular" reaction.

Absorption of Patiria fertilizin by treated and normal sperm. If normal *Patiria* sperm are "univalent" with respect to exposed antifertilizin groups, the cells must

be made multivalent before they can be expected to agglutinate. The adjuvant is believed to effect such a conversion to the multivalent form by "exposing" latent or unreactive antifertilizin present on or near the sperm surface. Treated sperm then should bind more fertilizin than the normal "univalent" sperm. One of three absorption experiments demonstrating this is recorded in Table III.

TABLE III

Absorption of fertilizin by sea water and egg white treated Patiria *sperm*

Absorbing Mixtures			
Tube I	Tube II	Tube III	Tube IV
0.5 cc. sea water 0.5 cc. fertilizin 0.5 cc. sperm	0.5 cc. egg white 0.5 cc. fertilizin 0.5 cc. sperm	0.5 cc. egg white 0.5 cc. fertilizin 0.5 cc. sea water	0.5 cc. sea water 0.5 cc. fertilizin 0.5 cc. sea water

Titration of absorbed fertilizin solutions

Dilution of absorption supernatant	Tube I	Tube II	Tube III	Tube IV
1/2	+++	−	++++	++++
1/4	++	−	++++	++++
1/8	+	−	++++	++++
1/16	+	−	+++	+++
1/32	+	−	+++	+++
1/64	+	−	++	++
1/128	+	−	++	++
1/256	−	−	++	++
1/512	−	−	+	+
1/1024	−	−	+	+
1/2048	−	−	−	−

Four absorption tubes were prepared as indicated in the table. Fifty per cent sperm was used in the absorption and raw isotonic hen's egg white was employed as adjuvant. The tubes were refrigerated for nine hours to allow for complete reaction, and then centrifuged. The supernatants were then titrated for fertilizin with one per cent treated sperm. In absorption tubes III and IV sea water was substituted for the sperm added to tubes I and II. No adjustment was made in the titration for the volume of absorbing sperm removed from I and II by centrifugation. This is justified since the titration was made on a comparative basis and tubes III and IV represent controls for neutralization of fertilizin by egg white. Furthermore, the error in absolute values introduced by this involves something less than ⅙ of a dilution and therefore is well within the error of the method. Likewise, no adjustment was made in the supernatant of tube I for the adjuvant present in the absorption supernatant of tube II. Such adjustment was apparently unnecessary since the titers of the control tubes III and IV were the same (1024) The titers of these tubes also show that the egg white does not neutralize fertilizin. Comparison of tubes I and III shows that the sea water-sperm mixture caused an 8- to 64-fold drop in fertilizin concentration. However, in tube II (titer 0)

the adjuvant-sperm mixture completely exhausted the fertilizin. The striking difference in the titers of tubes I and II (128 and 0 respectively) demonstrates clearly that treated sperm has a greater fertilizin binding capacity than normal sperm.

Effect of the Adjuvant on the Fertilizing Power of Patiria Sperm

Since the adjuvant increases the fertilizin binding power of sperm and also the motility of these cells, it seemed likely that treated sperm would be unusually effective in fertilization. Several experiments comparing the treated and normal sperm in this respect showed this to be the case. The results of one such experiment are given in Table IV.

Table IV

The effect of hen's egg white on the fertilizing power of Patiria *sperm*

Sperm dilution	Egg white treated sperm	Sea water treated sperm	Egg white + Patiria eggs	Sea water + Patiria eggs
	% cleavage	% cleavage	% cleavage	% cleavage
1/2	94% (75)*	38% (95)*	0.7% (152)*	0.0% (118)*
1/4	95% (66)	0% (58)		
1/8	89% (45)	7.4% (54)		
1/16	88% (50)	6.8% (74)		
1/32	89% (53)	2.0% (65)		

* Total number of eggs counted.

A fresh one per cent sperm suspension was divided into two parts. One part was diluted serially (in twofold steps) with boiled isotonic hen's egg white. The other part was diluted similarly but with sea water. Sperm dilutions are given as the dilution of one per cent sperm added to the eggs. One drop of each sperm suspension was added to twelve drops of *Patiria* eggs in 6 cc. of sea water. To control for parthenogenesis one drop of egg white was added to one dish of eggs and a drop of sea water to a second dish. The eggs were examined for cleavage three hours after addition of sperm.

Although the number of eggs counted was small it can readily be seen that the egg white treatment greatly increased the fertilizing power of the sperm. Even at the lowest dilutions the treated sperm was twice as effective as the untreated cells. At high dilutions the treated sperm fertilized nearly 90 per cent of the eggs whereas the normal sperm fertilized less than 10 per cent. Gray (1915) has reported a similar result with alkali treated *Asterias glacialis* sperm.

Specificity of the Adjuvant

Although no exhaustive search was made for different sources of adjuvant, a number of unrelated preparations were encountered which stimulated *Patiria* sperm and rendered it agglutinable by fertilizin. These preparations included *Panulirus*, rabbit, fish (*Crassius*), and hen sera, and hen's egg white. Thus the source of the adjuvant is not highly specific.

Properties of the Egg White Adjuvant

The adjuvant action can not be attributed to the high pH of raw egg white (Needham, 1931) since the material is active at sea water pH. Therefore, preliminary attempts were made to characterize an "active principle" in the hen's egg white. The agent is quite heat stable. Its activity was retained even after several hours at 100° C. In fact heating increased the activity of the egg white several fold. Ultraviolet light had a similar effect. This suggests the release of inactive bound agent. The "active principle" was quite nondialyzable both before and after heating. It was soluble in saturated ammonium sulfate, but insoluble in strong acetone and alcohol. Thus it is probably neither ordinary protein nor lipoid.

Discussion

From the evidence presented it is concluded that fertilizin is obtained from *Patiria* eggs, and that this fertilizin, although it does not agglutinate normal sperm, is a multivalent agglutinin that reacts with the normal sperm. It is further believed that the exposed antifertilizin of normal *Patiria* sperm is limited to a small area of the sperm surface and contains only a few or even a single combining group complementary to fertilizin. For practical purposes such sperm may be considered "univalent." It is necessary to assume that some antifertilizin is exposed on the normal sperm to explain the absorption of fertilizin by such sperm and to account for the "granular" agglutination reaction. This then is a reversal of Tyler's (1941, 1942) view. He believed that the normal *Patiria* fertilizin was "univalent" and that the sperm was multivalent.

The various adjuvant solutions stimulate the sperm to intense motility and presumably expose more antifertilizin on the sperm surface. The latter effect is believed to be the essential one in rendering the sperm agglutinable. This action of the adjuvants bears a superficial resemblance to the "transformation" of human erythrocytes by an enzyme present in certain bacterial filtrates (Thomsen effect). Any human serum will agglutinate these transformed cells. There are several important differences between the process of erythrocyte transformation (Friedenrich, 1930) and the action on starfish sperm. The transformation requires a considerable period of time (15 minutes to several hours), is irreversible, and involves a fixation and subsequent release of the transforming principle. The action on *Patiria* sperm takes place very rapidly, the process reverses slowly when the adjuvant is removed, and it involves no fixation of the adjuvant. Repeated attempts failed to show any neutralization or absorption of egg white adjuvant by sperm or sperm-fertilizin mixtures. Friedenrich (1930) believes that a new agglutinogen is developed which is not present in latent or unreactive form on the normal erythrocyte. However, the case of *Patiria* sperm is more easily explained by assuming that a considerable amount of antifertilizin is in latent form on or near the cell surface.

Di Macco's (1923) "coagglutination" of sheep erythrocytes by mixtures of ricin and guinea pig serum also resembles the fertilizin-adjuvant agglutination of *Patiria* sperm. Neither ricin nor guinea pig serum alone agglutinated the sheep cells. Absorption of the separate solutions with cells failed to remove the active agents. Agglutination failed to occur if the ricin and guinea pig serum were mixed

first and the sheep cells added subsequently. Thus neither of the necessary agents reacted directly with the cells. Di Macco concluded that agglutination of sheep cells resulted from a reaction between the cells and an evanescent ricin-serum complex formed at a critical stage in the reaction between these substances. It is apparent, then, that the mechanism of the coagglutination is fundamentally different from the fertilizin-adjuvant agglutination of *Patiria* sperm.

The striking difference in fertilizing power of normal and adjuvant-treated sperm can be explained by the motility of the cells. Furthermore, this effect should be expected, regardless of motility, from the recent views of Tyler (1941). He has shown that fertilizin treatment lowers the fertilizing power of *Patiria* sperm and explained this by assuming that at fertilization a union occurs between antifertilizin on the sperm and fertilizin at the egg surface. If all of the sperm antifertilizin is bound by free fertilizin, then no reaction can occur between sperm and the surface of the egg. It follows from this that the normal univalent sperm would have much less chance of reaching the egg surface in an unsaturated condition than would the multivalent sperm. At present it is impossible to judge the relative importance of the intense motility and the multivalency of the adjuvant-treated sperm in this fertilization effect. If this increased fertilizing power should be found in species that regularly give low percentages of fertilized eggs, it might be useful for technical purposes.

Summary

I. Starfish sperm does not ordinarily agglutinate when treated with homologous fertilizin. However, when the sperm of some species (*Patiria miniata, Pisaster ochraceus, Astropecten* sp.) is treated with certain adjuvants the cells become intensely active and agglutinate when fertilizin is added. This reaction provides a means for studying the relationship between starfish sperm and fertilizin.

II. *Patiria* sperm will combine with homologous fertilizin and remove it from solution even in the absence of the adjuvant.

III. Cross tests between *Patiria, Pisaster* and *Astropecten* sperm and fertilizin solutions revealed no cross agglutination reactions.

IV. It is concluded that *Patiria* fertilizin is multivalent, since irradiated fertilizin will not agglutinate treated sperm but will inhibit the agglutination of such sperm by normal fertilizin; and since normal fertilizin will agglutinate sperm which has been freed of adjuvant.

V. It is suggested that normal *Patiria* sperm possesses but a single antifertilizin combining group and that more such groups are exposed on the sperm surface through the action of the adjuvant. Experiments which show that the fertilizin binding power of sperm is increased by the adjuvant support this view.

I am most grateful to Dr. Albert Tyler for the aid and encouragement he has given throughout the course of the work.

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